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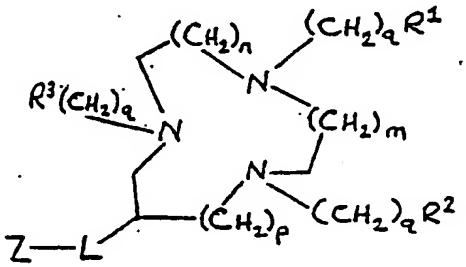
(54) Title: TRI-AZA MACROCYCLES AND METAL COMPLEXES THEREOF



(I)

(57) Abstract

Tri-aza macrocycles of formula (1), wherein m and n, which may be the same or different, is each zero or an integer 1, 2, or 3; p is zero or an integer 1 or 2; q is zero or an integer from 1 to 6 inclusive; R^1, R^2 and R^3, which may be the same or different, is each a hydrogen atom or an alkyl, alkoxyalkyl, $-CO_2H$, $-SO_3H$, PO_3H_2 or aryl group; L is a covalent bond or linker group; Z is a hydrogen atom or a reactive functional group, with the proviso that when L is a covalent bond Z is a reactive functional group; and metal complexes and/or salts thereof are described together with processes for their preparation and compositions containing them. The compounds are useful for imaging and in the treatment of abnormal cell disorders, such as in the treatment of tumours, and are particularly suitable for coupling to other molecules such as proteins for use in diagnosis and therapy.

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TRI-AZA MACROCYCLES AND METAL COMPLEXES THEREOF

Field of the Invention

This invention relates to functionalised tri-aza macrocycles, to metal complexes thereof, to conjugate compounds containing the functionalised tri-aza macrocycles and metal complexes thereof and to their use in diagnosis and therapy.

Background to the Invention

The attachment of metal ions to proteins, peptides and other, smaller molecules is a fast expanding technology, which has numerous proven and potential applications in research, in industry and, particularly, in medicine.

In recent years, much of the impetus behind the development of this technology has been the ability to link metal ions to antibodies, especially monoclonal antibodies. Such metal labelled antibodies have found a widespread use, especially in medicine, where they have been employed, for example, to target the metal ion to a specific tissue type, both *in vitro* and *in vivo*. Thus, metal labelled antibodies have applications in locating specific tissue types (e.g. employing computer-aided tomographic techniques where the metal ion is in some way detectable) and in the treatment of cell disorders (e.g treating mammalian tumours where the metal ion is a cytotoxic radionuclide).

Conventionally, attachment of the metal ion to a protein such as an antibody has been achieved by complexation by an acyclic chelate such as a substituted diethylenetriaminepentaacetic acid [Gansow O. A. *et al*, Inorg. Chem., (1986), *25*, 2772] or ethylenediaminetetraacetic acid [Meares, C. F. *et al*, Acc. Chem. Res., (1984), *17*, 202] covalently linked to the antibody. Such acyclic complexes however tend to be unstable *in vivo* either as a

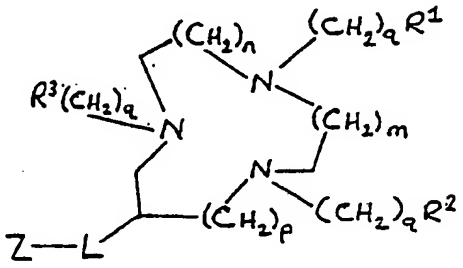
result of acid-catalysed decomplexation or competitive chelate binding by Ca^{2+} or Zn^{2+} in serum, or as a result of competition from transferrin [Moerlein, S. M. et al, Int. J. Nuc. Med. Biol., (1981) 8, 277]. The lack of stability can result in uncomplexed metal atoms in the body which have a cytotoxic effect on healthy tissue (e.g. bone marrow) or which markedly reduce the signal-to-noise ratio of an imaging technique.

A possible alternative to the use of acyclic chelates in the labelling of antibodies is the use of macrocyclic ligands, which has been suggested in broad terms [Gansow O.A. et al. Am. Chem. Soc. Symp. Ser., (1984), 241, 215; UK Patent Specification Publication No. 2122641; and Moi M. K. et al, Anal. Biochem., (1985), 148, 249-253].

We have now found a new class of functionalised tri-aza macrocyles, members of which are able to form more kinetically inert complexes with metal ions than are chelating agents conventionally in use for the attachment of metal ions to proteins and other molecules. The macrocycles of the invention are particularly useful for attachment to proteins, especially antibodies, to provide conjugate compounds capable of binding metals to give complexes which are advantageously stable in vivo.

Summary of the Invention

Thus, according to one aspect of the present invention we provide a compound of general formula (1):



(1)

wherein

m and n, which may be the same or different, is each zero or an integer 1, 2, or 3;

p is zero or an integer 1 or 2;

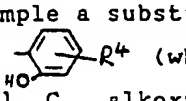
q is zero or an integer from 1 to 6 inclusive;

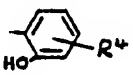
R^1, R^2 and R^3, which may be the same or different, is each a hydrogen atom or an alkyl, alkoxyalkyl, $-CO_2H$, $-SO_3H$, $-PO_3H_2$ or aryl group;

L is a covalent bond or a linker group;

Z is a hydrogen atom or a reactive functional group, with the proviso that when L is a covalent bond Z is a reactive functional group;

and metal complexes and/or salts thereof.

In the compounds of formula (1), alkyl groups represented by R^1, R^2 and R^3 may be for example C_{1-6}alkyl groups such as methyl or ethyl groups. Alkoxyalkyl groups represented by R^1, R^2 or R^3 may be for example C_{1-3}alkoxyC_{1-3}alkyl groups e.g. methoxymethyl. When R^1, R^2 or R^3 is an aryl group it may be for example a substituted phenyl group, such as a group of formula  (where R^4 is a hydrogen atom or a C_{1-6}alkyl, e.g. methyl, C_{1-3}alkoxy$_{1-3}$alkyl, e.g. methoxymethyl, or C_{6-12}aryl, e.g. phenyl group).

In general, compounds of formula (1) in which R^1, R^2 and R^3 are the same are preferred. Compounds of this type in which q is an integer from 1 to 6 inclusive, particularly an integer 1, and R^1, R^2 and R^3 are $-SO_3H$, $-PO_3H_2$,  or, in particular, $-CO_2H$, are especially preferred.

In the compounds of formula (1), it will be appreciated that the nature of the group L when it is a linker group may be varied widely without substantially affecting the usefulness of compounds of formula (1) and the metal complexes thereof. Thus L may be any suitable organic radical and may be for example an optionally substituted aliphatic hydrocarbyl chain, optionally interrupted by one or more heteroatoms selected from -O- or -S- or by one or more $-N(R^5)-$ (where R^5 is a hydrogen atom or a C_{1-6}alkyl group), $-CON(R^5)-$, $-N(R^5)CO-$, cycloaliphatic, aromatic, or heteroaromatic groups.

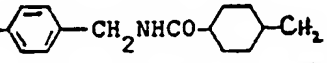
In the above definition, and in the same context whenever it appears below, the term "interrupted by" as applied to cycloaliphatic or aromatic groups is to be understood to also mean that these particular groups may additionally be present linked to the terminal carbon atom of the hydrocarbyl chain represented by L, at the opposite end of the chain to the carbon atom attached to the macrocycle.

Thus, for example, L may be an optionally substituted straight or branched C_{1-20}alkylene, C_{2-20} alkenylene, or C_{2-20} alkynylene chain, optionally interrupted by one or more -O- or -S- atoms or C_{5-8}cycloalkylene (e.g. cyclopentylene or cyclohexylene), C_{6-12}aromatic (e.g phenylene or substituted phenylene), C_{5-10}heteroaromatic (e.g furanyl, pyridyl), $-N(R^5)-$, $-CON(R^5)-$ or $-N(R)^5CO-$ groups.

Examples of substituents which may be present on the chain L include halogen atoms, e.g. fluorine, chlorine, bromine, or iodine atoms or groups selected from C_{1-6}alkoxy (e.g methoxy or ethoxy), hydroxy, nitro, $-N(R^6)(R^7)$, [where R^6 is a hydrogen atoms or a C_{1-6}alkyl group and R^7 is a C_{1-6}alkyl group; e.g. $-NHCH_3$ or $-N(CH_3)_2$], or substituted amido, e.g. a group of formula $-(CH_2)_nCON(R^8)(R^9)$ [where n is zero or an integer 1 to 4 inclusive, R^8 is a hydrogen atom or a C_{1-6}alkyl group, e.g. methyl and R^9 is an optionally substituted C_{1-6}alkyl group].

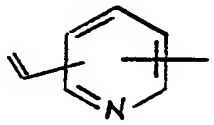
Substituted alkyl groups represented by R^9 include for example C_{1-6}alkyl groups substituted by one or more halogen atoms, or nitro, amino or hydroxy groups.

In general, in compounds of formula (1) the linker group is preferably an optionally substituted C_{1-10}alkylene, (especially C_{1-6}alkylene such as methylene, ethylene, propylene butylene, pentylene or hexylene) C_{2-10} alkenylene or C_{2-10} alkynylene chain optionally interrupted by one or more -O- or -S- atoms or cyclohexylene, phenylene, substituted phenylene, -NH-, $-N(CH_3)-$, -CONH-, $-CON(CH_3)-$ -NHCO- or $-N(CH_3)CO-$ groups.

Particular examples of linker groups represented by L include, for example, $-(CH_2)_d-$ (where d is an integer 1 to 4 inclusive), $-(CH_2)_d$  , $-(CH_2)_d$  , $-(CH_2)_dNHCO(CH_2)_e-$ (where e is an integer 1 to 4 inclusive) and $-(CH_2)_dNHCO(CH_2)_eOCH_2-$.

The reactive functional group represented by Z in compounds of formula (1) may be any group capable of reacting with a thiol, amino, carboxyl, hydroxyl, aldehyde, aromatic or heteroaromatic group. Aromatic groups include, for example, phenolic groups. Heteroaromatic groups include for example imidazolyl groups.

Thus, Z may be, for example, a halogen atom, for example a chlorine, bromine or iodine atom, or a group selected from -SH, $-NH_2$, hydrazine ($-NHNH_2$) or a derivative thereof, [for example $-N(CH_3)NH_2$, $-NHCONHNH_2$, $-NHCSNHNH_2$, or phenyl hydrazine], -NCO, -NCS, $-COR^{10}$, [where R^{10} is a halogen atom such as a chlorine or bromine atom, or a N_3, C_{1-6}alkoxy, e.g. methoxy, C_{6-12}aryloxy (e.g. nitrophenyloxy or dinitrophenyloxy), imidyloxy (e.g. succinimidyloxy) or imidazolyoxy group], imide, e.g. maleimide, a vinyl group of formula $-Het^1-C(Het^2)=CH_2$ (where Het^1 and Het^2, which may be the same or different, is each a nitrogen containing heterocyclic group, e.g. a pyridyl group or Het^1 is a nitrogen containing heterocyclic group and Het^2 is a hydrogen atom), for example a vinyl pyridyl group of formula



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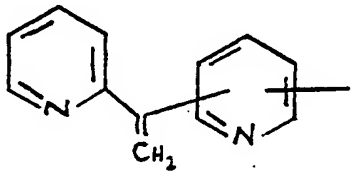

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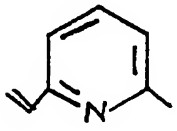

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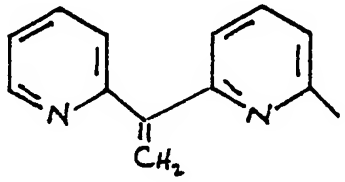

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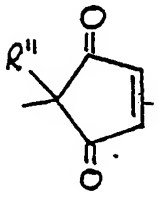
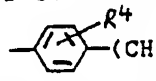


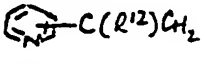
(where R^{11} is a C_{1-4}alkyl e.g. methyl, group).

Metal complexes of the compounds of formula (1) include complexes wherein the metal is di- or tripositive and has a coordination number from 2 up to 6, especially 6. Examples of such metals include indium (In), gallium (Ga), cobalt (Co), copper (Cu), lead (Pb) and bismuth (Bi). In, Ga, Co and Cu are preferred, particularly In and Ga. In general the metal is preferably a radioactive isotope. Indium, especially ^{111}In, is particularly preferred.

In general, optimum binding of the metal to the compounds of formula (1) may be achieved by selection of the ring size and where appropriate by adjusting the potential coordination number by choice of the group $-(CH_2)_qR^1$, $-(CH_2)_qR^2$, and/or $-(CH_2)_qR^3$ Thus a particularly important class of compounds of formula (1) is that wherein p is zero. Especially useful compounds are those wherein p is zero, m is an integer 1 and n is an integer 1. In general, compounds of formula (1) in which $-(CH_2)_qR^1$, $-(CH_2)_qR^2$ and $-(CH_2)_qR^3$ is each $-CH_2CO_2H$ are particularly useful.

Salts of the compounds of formula (1) include salts with bases, e.g. sodium or potassium salts, or acid addition salts such as hydrobromides or hydrochlorides. Pharmaceutically acceptable salts are particularly preferred.

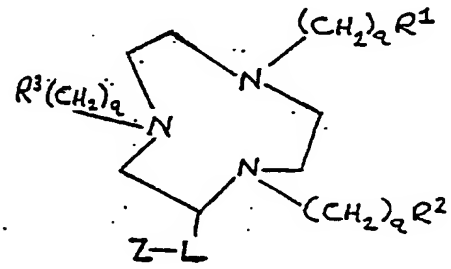
A particularly useful group of compounds of the invention has the formula (1) wherein R^1, R^2, R^3, m, n, p and q are as defined for formula (1) and the groups -L and Z together represent a group (1) $-(CH_2)_r-X-Y$ [where r is zero or an integer from 1 to 6 inclusive, X is a group -NH-,  (where R^4 is a previously defined and s is zero or an integer 1 to 4 inclusive), $-(CH_2)_SNHNH-$ or $-(OCH_2CH_2)_tNH-$ (where t is an integer 1 to 6 inclusive) and Y is

a group $-COZ^1$ or $-CO(R)Z^1$ (where R is a spacer group, and Z^1 is a group $-(CH_2)_tHal$ (where Hal is a halogen atom),  or  (where R^{12} is a nitrogen containing aromatic heterocyclic group, for example a pyridyl group)], or (2) a group $-(CH_2)_rNCS$; and the metal complexes and/or salts thereof.

In compounds of this type, the spacer group R may be for example an alkylene, e.g. ethylene, alkoxyalkylene, e.g. methoxymethylene, aryl, e.g. phenylene, aralkylene, e.g. phenalkylene such as phenethylene, or cycloalkylalkylene, e.g. cyclohexylmethylene group.

A further particularly useful group of compounds according to the invention has the formula (1) wherein R^1, R^2, R^3, m, n, p and q are as defined for formula (1) and the groups L and Z together represent a group $-(CH_2)_rXH$ (where r and X are as defined above) and the metal complexes and/or salts thereof.

An important group of compounds according to the invention has the formula (1a):



(1a)

wherein R^1, R^2, R^3, L and Z are as defined for formula (1) and metal complexes and/or salts thereof.

Compounds of this type in which R^1, R^2 and R^3 is each $-CO_2H$ are particularly preferred.

Compounds of formula (1a) in which L is a linker group [particularly those specfically identified for compounds of formula (1)] are especially useful.

Z in compounds of formula (1a) is preferably a reactive functional group, [particularly those specifically identified for compounds of formula (1)], especially a group of formula $-Het^1-C(Het^2)=CH_2$ or a dione of formula 

Indium complexes of the compounds of formula (1a) are particularly useful.

The compounds of formula (1) and the metal complexes and/or salts thereof have a diagnostic use as imaging agents in vitro and in vivo. The compounds of formula (1) and the metal complexes and/or salts thereof are also cytotoxic agents and may be used in the treatment of abnormal cell disorders, for example in the treatment of tumours.

For application of the compounds of formula (1) as imaging or cytotoxic agents, it is generally preferable to couple the compounds to other molecules such as proteins, especially antibodies, peptides or carbohydrates to form conjugate compounds, and the compounds of formula (1) are particularly well adapted for use in this respect.

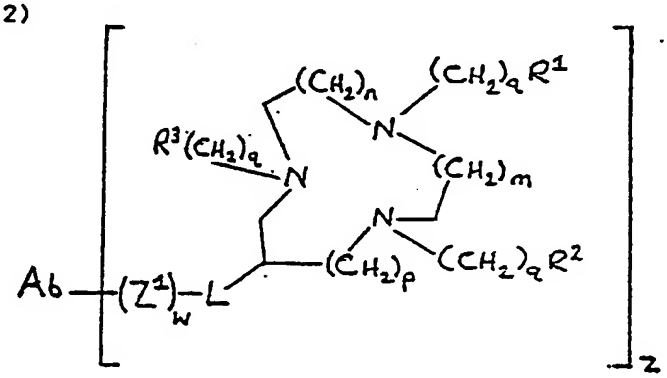
Thus, according to a further aspect of the invention, we provide a conjugate compound which comprises a compound of formula (1), or a metal complex and/or salt thereof, coupled to a protein, peptide or carbohydrate.

The compound of formula (1) may be coupled through any thiol, amino, carboxyl, hydroxyl, aldehyde, aromatic or heteroaromatic group present in the protein, peptide or carbohydrate.

In a preferred aspect of the invention, we provide a conjugate compound which comprises a compound of formula (1) or a metal complex and/or salt thereof, coupled to an antibody.

It is to be understood that conjugate compound according to the invention may contain more than one molecule of a compound of formula (1) coupled to any one protein, peptide or carbohydrate molecule.

In a particular aspect, the invention provides conjugate compound of formula (2)

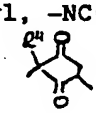


(2)

wherein m, n, p, q, R^1, R^2, R^3, and L are as defined for formula (1);

Z^1 is the residue of a reactive functional group;
w is zero or an integer 1;
z is an integer 1 or more;
Ab is an antibody; and metal complexes and/or salts thereof.

In the compounds of formula (2), the residue of a reactive functional group represented by Z^1 may in general be the residue of a reactive functional group Z as defined for formula (1).

In particular, Z^1 may be for example -S-, -NH- -NHN=, $-N(CH_3)N=$, -NHCONHN=, -NHCSNHN=, -N(Ph)N= (where Ph is phenyl, -NC(O)-, -NC(S)-, -CO-,  , $-Het^1-C(Het^2)CH_2-$ or 

The antibody Ab in the conjugates of formula (2) may be a complete antibody molecule or a fragment thereof, or an analogue or either of these, provided that the antibody comprises of a specific binding region. Thus the antibody may be polyclonal, or, preferably,

monoclonal, or a fragment thereof for example a Fab' or $F(ab)_2'$ fragment. If desired the antibody may be a recombinant antibody, (i.e. an antibody which has been produced using recombinant DNA techniques). The antibody may be a chimaeric antibody comprising linked antibody fragments, each from a different source (see for example International Patent Specification No. WO 86/01533).

The antibody may be specific for any number of antigenic determinants, but is preferably specific for one antigenic determinant. Particular determinants include tumour cell-associated antigens, particularly mammalian tumour cell antigens for example oncofetal antigens such as carcinoembryonic antigen or alphafetoprotein.

A particular useful antibody is that known as B72.3 [Colcher, D. et al Proc. Nat. Acad. Sci. USA (1981), 78, 3199].

The antibody Ab will in general be coupled to the remainder of the conjugate of formula (2) (i.e. the macrocycle and linker) through any appropriate reactive atom or group, for example a nitrogen or, especially, sulphur atom, present in the antibody. It will be appreciated that any one antibody molecule may contain more than one reactive group capable of coupling with the macrocycle and linker. Thus, for example, z in the conjugates of formula (2) may be an integer 1, 2, 3, 4, 5, 6 or more depending on the number of macrocycles linked to any particular antibody molecule or fragment or analogue thereof.

Indium complexes of conjugates of formula (2) are particularly useful.

It is to be understood that the definitions and preferences expressed for m, n, p, q, R^1, R^2, R^3 and L in compounds of formula (1), and for classes of compounds of formula (1) are also applicable to conjugates of formula (2).

Particularly useful conjugate compounds according to the invention are those comprising a compound of formula (1a), or a metal complex and/or salt thereof, coupled to an antibody. The indium complexes of these conjugates are especially important.

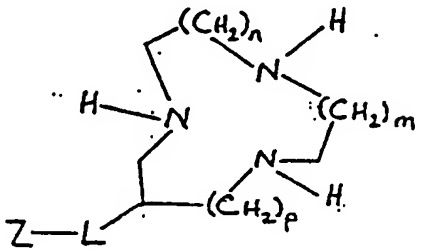
The compounds of formulae (1) and (2) may be formulated for use in accordance with conventional practice, and thus according to a further aspect of the invention we provide a composition comprising a compound of formula (1) or a compound of formula (2) or a metal complex and/or salt thereof, together with one or more pharmaceutically acceptable carriers.

Particularly suitable compositions according to the invention are those adapted for parenteral administration, especially intravenous administration. Suitable formulations of this type include solutions of the compounds of formulae (1) or (2) in isotonic saline.

The quantities of compounds of formulae (1) or (2) used in formulations according to the invention will vary according to the intended use (i.e. imaging or therapy) and other variables such as the intended cell target, but may be easily determined in accordance with conventional practice for reagents of this type.

Compounds of the invention may be prepared by the following processes wherein the groups and symbols R^1, R^2, R^3, m, n, p, q, L, Z, Ab and z are as defined for formulae (1) and (2) except where stated otherwise. Where a metal complex is desired as a final product, the complexation with a metal atom may be carried out as a final step in the production process, as described below for the complexation of compounds of formulae (1), or alternatively it may be desirable to complex the metal at an earlier stage in the process, providing of course that the requisite macrocycle structure is present. In the following processes, it may be desirable to use starting materials in which the group Z is in a protected state, or which contain a precursor of the group, as discussed below.

Thus, according to a further aspect of the invention a compound of formula (1) or a metal complex thereof may be prepared by reaction of a corresponding compound of formula (3)



(3)

or a metal complex thereof, with a reagent $R^1(CH_2)_qD$ (where D is a displaceable group). Displaceable groups represented by D include for example halogen atoms, for example a bromine, chlorine or iodine atom.

The reaction may be performed in a solvent such as water or an organic solvent such as a nitrile e.g. acetonitrile or an alcohol e.g. isopropanol or an amide e.g. dimethylformamide in the presence of a base, e.g. an inorganic base such as an alkali metal carbonate or hydroxide, e.g. sodium, potassium or caesium carbonate, or sodium, potassium or lithium hydroxide, at a high temperature e.g. the reflux temperature.

In this reaction, the group Z may need to be in a protected state. Conventional protecting groups may be used, depending on the nature of Z, and may be removed using standard procedures, once the desired reaction has been effected. Similarly, when the reagent $R^1(CH_2)_qD$ contains an acid group this may also need to be protected, for example as an ester e.g. a methyl ester. The acid may be re-generated after the desired reaction is complete, for example by hydrolysis using an acid such as sulphuric acid.

It will be appreciated that where it is desired to prepare a compound of formula (1) in which R^1, R^2 and R^3 are not the same this may be achieved by first selectively N-protecting the compound of formula (3) or a precursor using an appropriate amine protecting group(s), for example a p-toluenesulphonyl group as described below, in accordance with conventional practice. Reaction of the N-protected compound (3) with $R^1(CH_2)_qD$ followed by deprotection and further reaction as necessary with other reagents $R^1(CH_2)_qD$ then yields the desired compound in which R^1, R^2 and R^3 are not the same.

Where metal complexes of compounds of formulae (1) or (2) are required (or any other suitable macrocyclic intermediate described herein) these may be prepared by treating the compound with a metal salt (for example a metal halide) in an appropriate solvent for example an aqueous or non aqueous solvent, (e.g. acetonitrile, acetone, propylene carbonate, dimethylformamide or dimethylsulphoxide) at any suitable temperature from 0^oC to 100^oC such as 10^o to 80^oC e.g. around 60^oC.

In another process, a compound of formula (1) or a metal complex thereof wherein R^1, R^2 and R^3 is each $-(CH_2)_qPO_3H_2$ (where q is an integer 1 to 6) may be prepared by reaction of a compound of formula (3) or a metal complex thereof with phosphorous acid and an aldehyde R^bCHO (where R^b is a hydrogen atom or a C_{1-5}alkyl group) in the presence of an acid, such as hydrochloric acid at an elevated temperature, e.g. 100^o-130^oC.

Compounds of formula (1) may also be prepared by interconversion from other compounds of formula (1). Thus one functional group Z may be exchanged for another and, if desired a linker group L changed to another by appropriate manipulative reactions. For example, a compound of formula (1) where -L-Z is a group

$-L^1-NHCO-L^2-Z$ (where $-L^1-NHCO-L^2$ represents the group L) may be prepared by reaction of a corresponding compound wherein -L-Z represents $-L^1-NH_2$ with a reagent R^bO-L^2-Z (where R^b is for example an imide, such as succinimide, or a substituted phenyl group such as a p-nitrophenyl group) in the presence of a tertiary amine, such as diisopropylethylamine, in a solvent such as dimethylformamide.

Reagents of formula R^bO-L^2-Z are either known compounds or may be obtained form known starting materials using methods analogous to those used for the preparation of the known compounds.

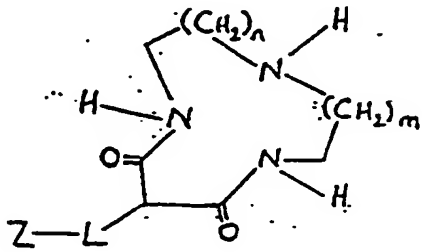
A conjugate compound of formula (2) or a metal complex thereof may be prepared by reaction of a corresponding compound of formula (1) or a metal complex thereof with an antibody Ab (as previously defined).

The reaction may be performed in a suitable solvent, for example an aqueous solvent such as a phosphate buffer, at an appropriate temperature, for example at 0^o-30^oC, especially 0^o-10^oC e.g. 4^oC.

The antibody Ab may be obtained using procedures well known in the art. If desired, before the coupling reaction, the antibody may first be treated to yield appropriate groups for reaction with the compound of formula (1). Thus for example the antibody may be subjected to oxidation, for example periodate oxidation to yield aldehyde groups, or, in particular, may be treated with a reagent [e.g. Traut's reagent (2-iminothiolane)] using standard procedures to generate free sulphydryl groups in the molecule.

Salts of compounds of formulae (1) or (2) and their metal complexes may be prepared by conventional means, for example by reaction with an appropriate base or acid in a suitable aqueous solvent.

Intermediates of formula (3) in which p is an integer 1 may be prepared by reduction of a diamide of formula (4):



(4)

(where R^{13} is a hydrogen atom or a nitrogen protecting group, for example a p-toluenesulphonyl group) using a reducing agent such as borane in a solvent such as tetrahydrofuran at a high temperature e.g. the reflux temperature, followed by treatment with an acid such as hydrochloric acid and, where necessary, followed by removal of the protecting group.

Intermediate diamides of formula (4) may be prepared by reaction of a diamine of formula (5):

$$H_2N\text{-}CH_2(CH_2)_nNR^{13}(CH_2)_mCH_2NH_2 \qquad (5)$$

with a diester of formula (6):

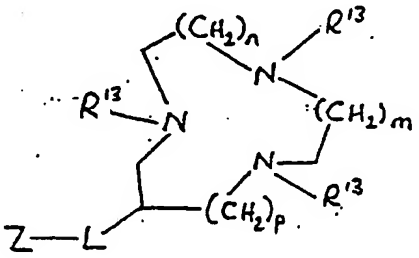
$$R^{14}O_2CCH(L\text{-}Z)CO_2R^{14} \qquad (6)$$

(where R^{14} is for example an alkyl group such as a methyl or ethyl group) in a solvent such as ethanol at reflux temperature, followed where appropriate by reaction with a reagent to introduce the protecting group R^{13} (e.g. by reaction with p-toluenesulphonyl chloride in a solvent such as dichloromethane in the presence of a base such as triethylamine at e.g. reflux).

It will be appreciated that in the above reactions to obtain an intermediate of formula (3) the group Z may need to be in a protected form. Alternatively a precursor of the group may be used. For example where Z is an amino group, the amino function may be generated from a corresponding nitrile during the reduction of a diamide of formula (6). The starting material for this reaction may be prepared from a compound of formula (6), in which Z is a nitrile group, as described previously.

The intermediate compounds of formula (3) and (4) are novel and form further aspects of the invention.

Intermediates of formula (3) in which p is zero or an integer 2 may be prepared by deprotection of a compound of formula (7)



(7)

(where p is as just defined, R^{13} is a protecting group as defined above and -L-Z is as defined previously). The deprotection will depend on the nature of the protecting group R^{13}. Thus, for example, when R^{13} is a p-toluenesulphonyl group removal of this may be achieved by treatment of the compound of formula (7) with an acid, for example HBr-acetic acid, in the presence of phenol at a high temperature, or by reaction with lithium in liquid ammonia in the presence of an alcohol such as ethanol.

Intermediates of formula (7) may be prepared by treating a compound of formula (8)

$$R^{13}NHCH_2CH(L\text{-}Z)(CH_2)_pN(R^{13})CH_2(CH_2)_mNHR^{13} \quad (8)$$

with a compound $R^{13}OCH_2(CH_2)_nOR^{13}$ in the presence of a base such as caesium carbonate in a solvent such as dimethylformamide.

Intermediates of formula (8) may be prepared by reduction of compounds of formula (9)

$$H_2NCOCH(L\text{-}Z)(CH_2)_pNHCO(CH_2)_mNHR^{13} \quad (9)$$

using for example borane as described above with intermediates of formula (4), followed by protection using a suitable protecting agent, for example p-toluenesulphonyl chloride as described previously.

Intermediates of formula (9) may be prepared by reacting an appropriate substituted amino acid of formula (10)

$$R^{11}O_2CCH(L\text{-}Z)(CH_2)_pNH_2 \quad (10)$$

(where R^{11} is a previously defined) with a reagent $HalOC(CH_2)_mNHR^{13}$ (where Hal is a halogen atom) in the presence of a base such as triethylamine, followed by reaction with ammonia in a solvent such as methanol.

The intermediates of formula (10) are either known compounds or may be prepared by methods analogous to those used for the preparation of the known compounds.

In an alternative process, intermediates of formula (7) in which p is zero may be prepared by reaction of a compound of formula (11)

$$R^{13}HNCH(L\text{-}Z)CH_2N(R^{13})CH_2(CH_2)_mNHR^{13} \quad (11)$$

with a compound $R^{13}OCH_2(CH_2)_nOR^{13}$ in the presence of a base such as caesium carbonate in a solvent such as dimethylformamide.

Intermediates of formula (11) may be prepared by reduction of compounds of formula (12)

$$H_2NCH(L\text{-}Z)CONHCH_2(CH_2)_mNHR^{13} \qquad (12)$$

using for example borane as described above, followed by reaction to introduce the protecting group R^{13}, for example with p-toluenesulphonyl chloride as described previously.

Intermediates of formula (12) may be prepared by reaction of an appropriately substituted amino acid of formula (13)

$$H_2NCH(L\text{-}Z)CO_2R^{14} \qquad (13)$$

(where R^{14} is as defined above with a diamine $H_2NCH_2(CH_2)_mNH_2$ at a high temperature, e.g. the reflux temperature.

Amino acids of formula (13) are either known compounds or may be prepared by methods analogous to those used for the preparation of the known compounds.

The invention is illustrated by the following Examples.

Description of Specific Embodiments

Intermediate 1

3-para-Cyanobenzyl-1,5,9-triaza-2,4-dioxocyclododecane

To a solution of p-cyanobenzyldiethyl malonate (5.50g) in dry ethanol (500ml) was added 1,7-diamino-4-aza-heptane (2.62g) and the mixture was refluxed for 5 days. After evaporation of solvent, the residue was chromatographed on silica [eluting with 1% NH_4OH, 40% methanol, 59% CH_2Cl_2] to yield the title compound as a colourless solid (990mg) m.p. 263-265°C m/e (chemical ionisation, NH_3) 315 (M^++1), 316 (M^++2); ∂_H ($CDCl_3$) 7.53 (2H,d), 7.50 (2H, br NHCO), 7.34 (2H, d), 3.65 (2H, mult.) 3.34 (2H,d), 3.23 (1H, mult.), 3.21 (2H, d), 2.9-2.8 (2H, mult.), 2.68 (2H, mult.) 1.73 (2H, mult.), 1.56 (2H+1H, mult.).

Intermediate 2

3-para-Cyanobenzyl-1,5,9-triaza-5-p-toleunesulphonyl-2,4-dioxocyclododecane

To a suspension of Intermediate 1 (628mg) in dichloromethane (20ml), was added triethylamine (200mg) and p-toluenesulphonyl chloride (381mg) and the mixture was heated at reflux for 18h. After filtration, the residue was chromatographed on silica (CH_2Cl_2-methanol) to yield the title compound as a colourless foam (749mg) m/e [desorption chemical ionisation (NH_3)] 469 (M^++1), 468, 289, 255, 215, 157. i.r. (KBr), 3300 (NH), 2215 (CN), 1670, 1640, 1530 (br.s.), 1160cm^{-1} ∂_H(CD_3OD), 7.74 (2H,d), 7.65 (2H,d), 7.43 (4H, mult.), 3.49-3.11 (9H,m.), 2.91-2.82 (2H,m), 2.47 (3H,s), 1.74 (2H,m), 1.60 (2H,m).

Intermediate 3

3-para-Aminomethylbenzyl-1,5,9-triaza-5-tosyl-cyclododecane

To a suspension of Intermediate 2 (468mg) was added 1M borane-tetrahydrofuran solution (20ml) and the mixture was refluxed for 36h. Excess borane was destroyed by slow addition of methanol (5ml) and solvents were removed under reduced pressure to yield a residue which was treated with 6M HCl (15ml) and refluxed for 3h. After removal of water, the residue was taken up in the minimum volume of 2M KOH solution and extracted with chloroform (3 x 20ml). Removal of solvent geve the title compound as a colourless oil (400mg). m/e (chemical ionisation, NH_3) 446 (M^++2), 445 (M^++1), 324, 289, 229. $\partial_H(CDCl_3)$ 7.67 (2H,d), 7.29 (2H,d), 7.23 (2H,d), 7.11 (2H,d), 3.83 (2H,s), 3.27-3.16 (4H,m), 2.86-2.77 (4H,m), 2.62-2.52 (4H,m), 2.48-2.45 (2H,d), 2.42 (3H,s), 2.17-1.91 (4H,m), 1.78-1.68 (4H,m). ∂_c ($CDCl_3$), 143.2, 129.6, 129.0, 127.4, 127.1, 51.8, 46.1, 45.9, 45.2, 38.3, 37.7, 26.6, 21.5, i.r. (Nujol) 3300 (NH), 1590, 1160cm^{-1}.

Intermediate 4

3-para-Aminomethylbenzyl-1,5,9,-triazacyclododecane tetrahydrobromide

To a solution of Intermediate 3 (400mg) in HBr-acetic acid (45%, 25ml) was added phenol (0.5g) and the mixture was heated at 110°C for 36h. After cooling a colourless solid was filtered, washed with acetone and dried (K_2CO_3) to yield the title compound, (525mg) i.r. (KBr) 3600-3300 (br,NH), 2890,1585cm^{-1} ∂_H (D_2O) 7.11 (2H,d), 7.06 (2H,d), 3.79 (2H,s), 3.13-2.78 (12H,m), 2.56 (2H,d), 2.29 (1H,m), 1.81-1.69 (4H,m).

Intermediate 5

3-para-Acetamidomethylbenzyl-1,5,9-triazacyclododecane

To an aqueous solution of Intermediate 4 (525mg pH6.8, 5ml) was added a solution of p-nitrophenylacetate (543mg) in dioxan (5ml) and the mixture was stirred at 35°C for 18h. After washing with ether (3 x 10ml) the pH of the solution was raised to 13.5, and the solution extracted with chloroform (5 x 10ml), dried (K_2CO_3), filtered and evaporated to give the title compound as a colourless oil (228mg), m/e (chemical ionisation, NH_3) 334 (M^++2), 333 (M^++1). ∂_H ($CDCl_3$) 7.25 (2H,d), 7.16 (2H,d) 4.39 (2H,d), 3.48 (2H,s), 2.87-2.53 (14H,m) 2.17 (1H,m), 2.02 (3H,s) 1.85-1.40 (7H,m).

Intermediate 6

3-para-Acetamidomethylbenzyl-N,N',N''-tricarboethoxymethyl-1,5,9-triazacyclododecane

To a solution of Intermediate 5 (166mg) in dry acetonitrile (5ml) was added anhydrous sodium carbonate (170mg) and ethyl bromoacetate (251mg) and the mixture refluxed for 18h. After filtration and removal of solvent, the residue was chromatographed on neutral alumina (activity II/III, 3% methanol/CH_2Cl_2) to yield the title compound as a colourless gum (59mg) m/e (chemical ionisation, NH_3) found 590.372253 (calculated 590.3679504) 591 (M^++1), 590 (M^+) 547, 503, 428. i.r. (liquid film) 3280 (NHCO), 2920, 1735, 1660cm^{-1}. $\partial_H(CDCl_3)$ 7.16 (4H,m), 5.80 (1H,m), 4.39 (2H,d), 4.13 (6H,q), 3.23 (4H,s), 3.19 (2H,s), 2.85-2.53 (8H,m), 2.45-2.23 (7H,m), 2.02 (3H,s). 1.78-1.60 (4H,m), 1.29-1.23 (6H, + 3H,t+t).

Intermediate 7

2,6-Diamino-1-hexanoic acid, ethylenediamine ester

2,6-Diamino-1-hexanoic acid, methyl ester, dihydrochloride (10.283g) was added (as solid) in small batches over a 50 minute period to ethylenediamine (100ml) at 90°C, with strirring. The temperature of the reaction mixture was then raised to 140°C for 6hrs, after which the ethylenediamine was removed by vacuum distillation to yield a brown residual oil which was taken up in 4M NaOH (25ml) and dried in vacuo. Methanol (30ml) was added, the solution was filtered, the methanol removed (Buchi) and the residue dissolved in CH_2Cl_2 (100ml), then filtered, and the filtrate rotovated down to give the title compound as a clear brown oil (8.827g). i.r (thin film) 3300/3280 3060 2930 2860 1650 1570 1470 1320cm^{-1}.

Intermediate 8

1,5,9-Triamino-3-aza-nonane, tetrahydrochloride

Intermediate 7 (3.754g) and borane-tetrahydrofuran (130mmol, 130ml) was refluxed for 21 hours. After removal of volatiles, the aminoborane was washed with methanol (2 x 100ml) and hydrolysed with 6M HCl (150ml, 110°C) for 3 hours. The resulting solution was evaporated, methanol (20ml) added and further evporated to yield the title compound (6.279g) as a white hygroscopic solid.

Intermediate 9

1,5-Diamino-(9-N-benzamidy)1-3-aza-nonane

Intermediate 8 (6.16g) and potassium hydroxide (4.4g) was dissolved in water (50ml) and, with stirring, copper carbonate (2.603g) was added. Continued stirring over 30 minutes at 50°C yielded an intense blue solution which was cooled to 0°C and benzoyl chloride 2.5ml added in 0.25ml portions over 90 minutes keeping the pH greater than 9 with periodic addition of KOH pellets. The solution was then allowed to stir at room temperature for 1 hour, then filtered and the filtrate treated with H_2S over 30 minutes. The solution was filtered once again to give a greeny-yellow filtrate which on addition of KOH to pH14 went a dark green, with a small amount of green precipitate. This was filtered off, the filtrate reduced in volume to 40ml and exhaustively extracted (13x) with CH_2Cl_2, dried (K_2CO_3), and evaporated to yield the title compound as a pale yellow oil (2.152g). ^{1}H-NMR (250MHz), $\delta(CDCl_3)$: 1.57 (m, 16H, CH_2, NH, NH_2) 2.37 (dd, 1H, CH), 2.67 (m 3H, CH_2N), 2.79(m, 3H, CH_2N).

Intermediate 10

1,5-Ditosylamino-3-tosyl-(9-N-benzamidyl)-3-aza-nonane

Intermediate 9 (1.978g) in dry CH_2Cl_2 (50ml) was added dropwise to a solution of tosyl chloride (5.087g), in dry CH_2Cl_2 (50ml) and the mixture was then allowed to stir for 2 1/2 hours at room temperature. The solution was then washed with water (20ml) dried (K_2CO_3), filtered and evaproated to an oily brown residue which was redissolved in CH_2Cl_2 (10ml). After a few minutes a white solid precipitated which was collected by filtration and washed with CH_2Cl_2 to give the title compound (1.701g).
TLC (silica; 5% methanol in CH_2Cl_2) Rf 0.44 m/e [desorption chemical ionisation (methanol)] 741 (M^++1), 740 (M^+).

Intermediate 11

2-(4-N-Benzamidyl)butyl-N,N',N''-tritosyl-1,4,7-triazacyclononane

Intermediate 10 (3.70g) was dissolved in anhydrous dimethylformamide (200ml) and caesium carbonate (3.26g) added under dry nitrogen. A solution of ethylene glycol ditosylate (1.85g), in anhydrous dimethylformamide (50ml) was slowly added, with stirring, over 4 hours. Stirring was continued overnight (20°C) and the temperature then raised to 65°C for 3 hours. The dimethylformamide was removed under reduced pressure and the residue dissolved in chloroform (200ml), washed with water (3 x 30ml) and dried (K_2CO_3). The residue was dissolved in the minimum volume of CH_2Cl_2 (15ml) and ethanol added slowly until turbidity. The flask was chilled to -20°C overnight and the title compound (2.6g) separated as a colourless solid. m/e [desorption chemical ionisation ($CHCl_3$)] : 767(M^++1), 766 (M^+) (100%).

Intermediate 12

2-(4-N-Benzamidyl)butyl-1,4,7-triazacyclononane

To Intermediate 11 (1.2g) in a flask under nitrogen was added ethanol (2ml), and liquid ammonia (100ml) then allowed to condense in the flask. Lithium metal (0.38g) was added and an intense blue colour developed which discharged within 20 minutes. After evaporation of NH_3 (4 hours) water (20ml) was added and the solution evaporated to dryness, taken up in 6 MHCl (20ml) washed with ether (3 x 20ml), evaporated to dryness and redissolved in 6M KOH (20ml) and extracted with dichloromethane (5 x 20ml). The extract was dried and evaporated to yield the title compound (360mg). m/e [desorption chemical ionisation (methanol)]: 305 (M^++1)

Example 1

3-para-Aminomethylbenzyl-1,5,9-triazacyclododecane-N,N'N''-triacetic acid

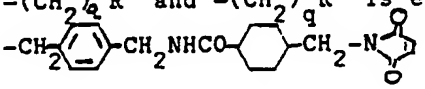
Intermediate 6 (59mg) was added to 6M HCl (5ml), and refluxed at 110°C for 18 hours, to yield the title compound following romoval of volatiles. The title compound was homogeneous by cation-exchange hplc, (Synchropak TSK DEAE, ammonium acetate (pH7), CH_3CN). m/e (FAB, glycerol) 465 (M^++1), 464 (M^+).

Example 2

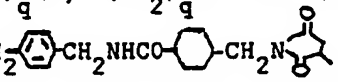
(a) The compound of Example 1 (2.6mg) in 0.5M 1,4-piperazine bis(ethanesulphonic acid) [PIPES; pH6.8, 300µl] was added to the sulphite salt of the n-hydroxysuccinimide ester of N-(4-carboxycyclohexylmethyl) maleimide [Yamada et al, Eur. J. Biochem., (1979). 101, 395; 10mg] in 1,4-dioxan (300ml). The reaction was monitored by high performance liquid chromatography (hplc) using a polymer reverse phase column (PLRP, 100Å, 15cm x 4.4mm internal diameter) using the following conditions:

TIME	%A	%B	%C
0	87	10	3
20	40	10	50
20.1	87	10	3

A = H_2O, B = 1M ammonium acetate pH6.5, C = CH_3CN.
λ = 268nm, Flow rate = 1.0ml/min.

The desired product eluted at 19 minutes and was purified preparatively to give a solution (approximately 5ml, 500µM) of a compound of formula (1) wherein m is 2, n is 2, p is 1, $-(CH_2)_qR^1$, $-(CH_2)_qR^2$ and $-(CH_2)_qR^3$ is each $-CH_2CO_2H$, and -L-Z is a group 

(b) The solution prepared in 2(a) was adjusted to pH5 using acetic acid/water (25%v/v) and to 90µl of this was added $^{111}InCl_3$ (10µl, 112µCi) and the reaction left at 55°C for 1 hour. The mixture was then purified by hplc using the above programme and radiometric detection. The ^{111}In complex of the compound of formula (1) described above eluted at 18 minutes and was collected and concentrated to a low volume (approximately 100µl).

(c) B72.3 monoclonal antibody [Colcher, D. et al Proc. Nat. Acad. Sci. USA (1981), 78, 3199; 1.2mg, previously modified with Traut's reagent to liberate 5.7 thiols/antibody] in 0.3M phosphate buffer (containing 2mM ethylenediaminetetraacetic acid; pH8.0; 110µl) was added to the ^{111}In complex prepared in 2(b) and the mixture was incubated at 4°C overnight then purified by PD-10 gel filtration chromatography to yield the ^{111}In complex of the conjugate of formula (2) wherein m is 2, n is 2, p is 1, $-(CH_2)_qR^1$, $-(CH_2)_qR^2$ and $-(CH_2)_qR^3$ is each $-CH_2CO_2H$, L-Z^1 is  , z is an integer 1 or more and Ab is a B72.3 monoclonal antibody.

The conjugate was injected in mice and the tissue distribution of the ^{111}In determined after 4 hours and 24 hours. The results are as shown in Table 1.

Table 1

Tissue	4 Hours *		24 Hours **	
	%Dose/g Tissue	% Total Injected Dose	% Dose/g Tissue	% Total Injected Dose
Blood	.32.4	60.9	21.275	47.52
Kidneys	12.32	2.88	8.92	2.495
Liver	8.605	12.36	6.555	9.955
Lungs	15.16	2.215	9.315	1.45
Spleen	6.075	0.41	6.44	0.53
Stomach	-	0.7	-	0.645
Small Intestine	-	2.39	-	2.6
Large Intestine	-	2.935	-	2.175

* Mean of 2 mice

** Mean of 2 mice

In control experiments using free ^{111}In and ^{111}In bound to macrocycle only, all radioactivity was cleared from the tissues in a few hours. The persistence of ^{111}In the tissues after 24 hours as shown in Table 1 illustrates that the indium complex described in 2(c) has been bound by the antibody.

Example 3

2-O-(6-ethenyl)-2-(pyridyl)methyl-N-[4-(2-perhydro-1,4,7-triaxaninine-1,4,7-tri(2-acetic acid)butyl)ethanamide].

(a) 2-(4-N-Benzamidyl)butylperhydro-1,4,7,-triazanonine-1,4,7-tri(2-acetic acid)

Lithium hydroxide monohydrate (14.5mg) and 2-bromoacetic acid (31.0mg) were added to Intermediate 12 (20mg) in water (0.476ml), the pH being kept at pH12 and above using additional lithium hydroxide as necessary. The solution was heated to 80°C and the reaction monitored by reverse phase high performance liquid chromatography using Lysl and the following programme:

BUFFERS: A = 0.1% trifluoroacetic acid/H_2O
B = 0.1% trifluoroacetic acid/CH_3CN

λ = 254nm; flow = 1.4ml/minute

Time (T; min)	%A	%B
0	95	5
20	5	95
25	5	95

Equilibration time = 10 minutes.

During the reaction a major peak built up within 30 minutes at T = 11.43 with a minor peak at T=12.077. The major peak was assumed to be the dicarboxylated species and further additions of acid were therefore made to the reaction solution over a 24 hour period to maximise the yield of the product at T = 12.077.

After 24 hours the reaction solution was subjected to preparative high performance liquid chromatography using Lys 1 to yield the title compound (100.9mg) of Part (a). m/e 479 (M^++1).

(b) 2-(4-Amino)butylperhydro-1,4,7-triazanonine-1,4,7-tri (2-acetic acid)

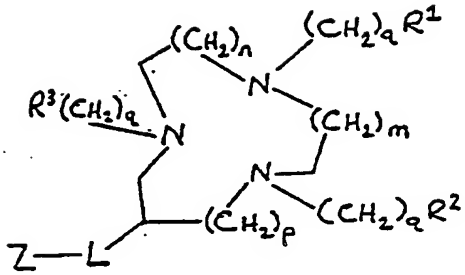
The compound of Part (a) [5mg] was dissolved in 6M HCl (3ml) and heated at 130°C under nitrogen for 18 hours. The solution was dried in vacuo and the residue redissolved in dry dimethylformamide. The drying and redissolving in dimethylformamide was repeated three times to yield the title compound of Part(b) which was used in the following reaction without further purification.

2-O-(6-ethenyl)-2-(pyridyl)methyl-N-[4-(2-perhydro-1,4,7-triazanonine -1,4,7-tri(2-acetic acid)butyl)ethanamide]

(c) The p-nitrophenyl ester of 2-vinyl, 6-methoxyacetic acid pyridine (3.0mg) in dry dimethylformamide (0.5ml) was added to the amine prepared in Part (b) above, followed by diisopropylethylamine (9.0mg). The reaction was monitored by reverse phase high performance liquid chromatography using Lys1 and the programme described in Part(a) above. The product which was observed at T=10.5min was collected and lyophillised to give the title compound [3.0mg] m/e (FAB, glycerol) 550 (M^++1). The title compound elutes at T=8.10 on anion exchange chromatography (Synchropak AX101) using the following programme:

CLAIMS

1. A compound of formula (1):



(1)

wherein

m and n, which may be the same of different, is each zero or an integer 1, 2, or 3;

p is zero or an integer 1 or 2;

q is zero or an integer from 1 to 6 inclusive;

R^1, R^2 and R^3, which may be the same of different, is each a hydrogen atom or an alkyl, alkoxyalkyl, $-CO_2H$, $-SO_3H$, $-PO_3H_2$ or aryl group;

L is a covalent bond or a linker group;

Z is a hydrogen atom or a reactive functional group, with the proviso that when L is a covalent bond Z is a reactive functional group;

and metal complexes and/or salts thereof.

2. A compound according to Claim 1 wherein q is an integer from 1 to 6 inclusive and R^1, R^2, and R^3 is each a $-CO_2H$ group.

3. A compound according to Claim 2 wherein q is an integer 1.

4. A compound according to any of the preceding claims wherein L is an optionally substituted aliphatic hydrocarbyl chain, optionally interrupted by one or more heteroatoms selected from -O- or -S- or by one or more $-N(R^5)-$ (where R^5 is a hydrogen atom or C_{1-6}alkyl group), $-CON(R^5)-$, $-N(R^5)CO-$, cycloaliphatic, aromatic, or heteroaromatic groups.

5. A compound according to any of the preceding claims wherein Z is a group capable of reacting with a thiol, amino, carboxyl, hydroxyl, aldehyde, aromatic or heteroaromatic group.

6. A compound according to Claim 5 wherein Z is a halogen atom or a group selected from -SH, $-NH_2$, hydrazine or a derivative thereof, -NCO, -NCS, $-COR^{10}$ (where R^{10} is a halogen atom or a N_3, C_{1-6}alkoxy, C_{6-12}aryloxy, imidyloxy or imidazolyloxy group), imide, $-Het^1-C(Het^2)=CH_2$ (where Het^1 and Het^2 which may be the same or different, is each a nitrogen containing heterocyclic group, or Het^1 is a nitrogen containing heterocylic group and Het^2 is a hydrogen atom) or a dione of formula  (where $R^{11}$is a C_{1-4} alkyl group).

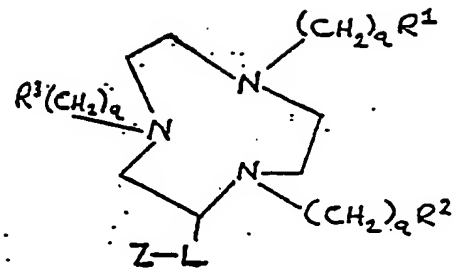
7. A metal complex of a compound of formula (1) as defined in any of the preceeding claims wherein the metal is di- or tripositvie and has a coordination number from 2 up to 6.

8. A metal complex according to Claim 8 wherein the metal is indium, gallium, cobalt, copper, lead or bismuth.

9. An indium complex of a compound of formula (1) as defined in Claims 1 to 7.

10. A compound according to any of the preceding claims wherein p is zero.

11. A compound of formula (1a)

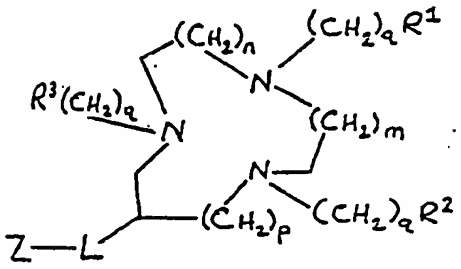


(1a)

wherein
R^1, R^2 and R^3, which may be the same of different, is each a hydrogen atom or an alkyl, alkoxyalkyl, $-CO_2H$, $-SO_3H$, $-PO_3H_2$ or aryl group;
L is a covalent bond or a linker group;
Z is a hydrogen atom or a reactive functional group, with the proviso that when L is a covalent bond Z is a reactive functional group; and metal complexes and/or salts thereof.

12. A compound according to Claim 11 wherein R^1, R^2 and R^3 is each a $-CO_2H$ group.

13. A conjugate compound which comprises a compound of formula (1):

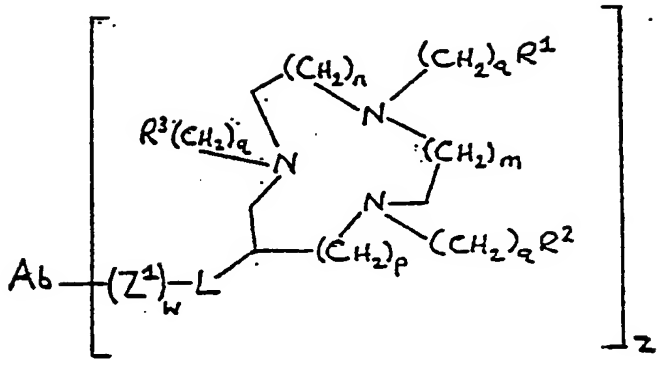


(1)

wherein

m and n, which may be the same of different, is each zero or an integer 1, 2, or 3;
p is zero or an integer 1 or 2;
q is zero or an integer from 1 to 6 inclusive;
R^1, R^2 and R^3, which may be the same of different, is each a hydrogen atom or an alkyl, alkoxyalkyl, $-CO_2H$, $-SO_3H$, $-PO_3H_2$ or aryl group;
L is a covalent bond or a linker group;
Z is a hydrogen atom or a reactive functional group, with the proviso that when L is a covalent bond Z is a reactive functional group;
or a metal complex and/or salt thereof, coupled to a protein, peptide or carbohydrate.

14. A conjugate compound of formula (2)



(2)

wherein

m and n, which may be the same of different, is each zero or an integer 1, 2, or 3;

p is zero or an integer 1 or 2;

q is zero or an integer from 1 to 6 inclusive;

R^1, R^2 and R^3, which may be the same or different, is each a hydrogen atom or an alkyl, alkoxyalkyl, $-CO_2H$, $-SO_3H$, $-PO_3H_2$ or aryl group;

L is a covalent bond or linker group;

Z^1 is the residue of a reactive functional group

W is zero or an integer 1

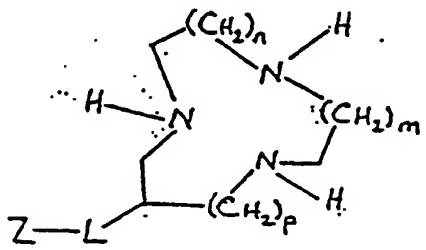
z is an integer 1 or more

Ab is an antibody; and metal complexes and/or salts thereof.

15. A composition comprising a compound of formula (1) as defined in Claim 1 or a metal complex and/or salt thereof together with one or more pharmaceutically acceptable carriers.

16. A composition comprising a compound of formula (2) as defined in Claim 14 or a metal complex and/or salt thereof with one or more pharmaceutically acceptable carriers.

17. A process for the preparation of a compound of formula (1) as defined in Claim 1 or a metal complex thereof comprising reacting a compound of formula (3)



(3)

[where m, n, p, L and Z are as defined for the compound of formula (1)] or a metal complex thereof with a reagent $R^1(CH_2)_qD$ [where D is a displaceable group and R^1 and q are as defined for the compound of formula (1)].

18. A process for the preparation of a conjugate compound of formula (2) as defined in Claim 14 comprising reacting a compound of formula (1) as defined in Claim (1) with an antibody Ab.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/00672

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 07 D 255/02; C 07 D 401/06; A 61 K 39/395; A 61 K 47/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 07 D 255/00; C 07 D 401/00; A 61 K 47/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	WO, A, 86/02352 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 24 April 1986, see pages 9-12 --	1,15-17
A	Chemical Abstracts, vol. 97, no. 24, 13 December 1982 (Columbus, Ohio, US) C. Bryden et al.: "Multinuclear NMR study of three aqueous lanthanide shift reagents: complexes with EDTA and two macrocyclic ligands", see page 617, abstract no. 206960z & Rare earths Mod. Sci. Technol. 1982, 3, 53-7 --	1
A	US, A, 4454106 (GANSOW et al.) 12 June 1984, see claims & GB, A, 2122641 (cited in the application)	1,8,13

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
3rd November 1988	28 NOV 1988
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8800672 SA 23705

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/11/88
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8602352	24-04-86	AU-A- 4869185	02-05-86
		EP-A- 0198051	22-10-86
		US-A- 4639365	27-01-87
		JP-T- 62501070	30-04-87
US-A- 4454106	12-06-84	FR-A- 2527928	09-12-83
		GB-A,B 2122641	18-01-84
		DE-A- 3320560	09-02-84
		JP-A- 59046227	15-03-84
		CA-A- 1225930	25-08-87

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82